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Acknowledgments

Acknowledgments should only be made to funding institutions and organizations and, if to persons, only to those who have made substantial contributions to the study.

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- References should be numbered consecutively (Arabic n merals) in the order in which they appear in the text. In the text section, the reference numbers should be given in parentheses. References within tables or legends should be numbered in accordance with the order in which they appear in the text.
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2- Books:

(a) Personal author: Speroff L, Glass RH, Kase NO. clinical gynecologic endocrinology and infertility. 4th edition, Baltimore, Williams & Wilkins; 1988: 105

(b) Chapter in book; Wilhelmsson L, Norstrom A, Tjugum 1, Hamberger L. Interaction between prostaglan dins and catecholamines on cervical collagen. In: Toppozada M., Bygdeman '. M., Hafez ESE, Eds. Prostaglandins and fertility regulation. Advances in reproductive health care. Lancaster, England, MTP Press Ltd., 1985 : 75 - 80.

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The Egyptian Journal of Fertility and Sterility has no page charges and offers no free reprints. The cost of printing illustrations in colour will be charged to the author(s). Significant changes in the printed proofs will also be charged to authors.

Contents :

Letter from the Editor:

Dear esteemed colleagues,

Warm greetings

We welcome your comments as well as the scientific activity to be incorporated in the upcoming issues. Very important subjects are included in this issue. During laparoscopic myomectomy, methylergonovine infusion greatly decreased loss of blood and the requirement for blood transfusions. Platelet rich plasma has the ability to improve clinical and biochemical pregnancy rate. Also, it has the ability to increase endometrial thickness in women with recurrent implantation failure. Prophylactic antibiotics before the surgical intervention of first-trimester miscarriage resulted in an insignificant decrease in postoperative pelvic infection. Cesarean scare defect repair significantly improves infertility and clinical pregnancy rate through laparoscopy. Pregnancy weight gain was associated with a significant effect on birth weight regardless of BMI. Additionally, maternal weight gain could be considered as a significant predictor of fetal weight. In women with secondary infertility and a residual myometrial thickness of less than 3 mm, hysteroscopic correction of a caesarean scar defect offers a minimally invasive method with a high success rate and no risks. Tranexamic acid significantly reduced intraoperative and postoperative blood loss after cesarean section and it can be safely used for prophylaxis against postpartum hemorrhage after cesarean section in low-risk patients. Sublingual administration of misoprostol before Mirena IUCD insertion could help to increase the ease of insertion with a significant decrease in the procedure time. Furthermore, it could improve patient satisfaction and decrease the pain experience.

Best regards.

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Ejaculation frequency improves ICSI outcomes for idiopathic Oligoasthenoteratozoosepmic patients
Running title: Ejaculation frequency and ICSI in iOAT

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Abstract

Objectives: We aimed to evaluate the association between increasing the frequency of ejaculation and ICSI outcomes for idiopathic oligoasthenoteratozoospermic (iOAT) male partners of couples undergoing ICSI.

Methods: The present prospective case-control study included 81 participants of iOAT men. The participants of the intervention group (n=44) received an instruction to change the lifestyle by increasing the ejaculation frequency and prescribed antioxidant therapy for 3months before ICSI. The subjects of the control group (n=38) received only antioxidant for 3 months before ICSI.

Results: A significant increase in the rate of top-quality blastocyst in the intervention group (42.9%) than in the control (24.7%), (RR: 0.76, 95% CI: 0.65-0.89, P=0.005) was detected. No significant differences in the rates of biochemical pregnancy (59% vs. 28.6%; RR: 1.2, 95% CI: 0.80–1.83), clinical pregnancy (52.3% vs. 37.8%; RR: 1.2, 95% CI 0.76–1.92), and implantation (37.8% vs. 27.9%; RR 1.4, 95% CI 0.88- 2.06] in the intervention group as compared to control were detected. Ongoing pregnancy rate was significantly higher in the intervention group than in the control group [RR 1.96, 95% CI 1.03-3.75; P=0.04).

Conclusions: High frequency of ejaculation may significantly improve the rates of the top quality blastocyst and ongoing pregnancy on ICSI/OAT cycles when combined with antioxidant therapy. Although the study's sample size is small to detect the clinical outcomes, there is a trend toward better rates of clinical pregnancy and implantation. However, a larger sample size is warranted to detect whether these would be of true significance.

Keywords: Idiopathic oligoasthenoteratozoospermia, Male factor, Ejaculation frequency, lifestyle modification, ICSI treatment.

List of abbreviation:

ART: Assisted reproductive technologies
ET: Embryo transfer
FSH: Follicle-stimulating hormone
GnRH: Gonadotropin-releasing hormone
hCG: Human chorionic gonadotropin
HMG: Human menopausal gonadotropin
ICSI: Intracytoplasmic sperm injection
IOAT: Idiopathic oligoasthenoteratozoospermia
IVF: In vitro fertilization
LH: Luteinizing hormone
MII: Metaphase II stage oocytes
rhCG: Recombinant human chorionic gonadotropin
ROS: Reactive oxygen species
SDs: Standard deviation
SPSS: Statistical Package for the Social Sciences program
WHO: World Health Organization

Introduction

There is one worrying certainty to have emerged this century and that is the increase, year on year, in male infertility with a decline in semen quality (1-3). Environment, nutrition and lifestyle factors are arguably the most significant cause of this phenomenon, even in the absence of conclusive evidence (4).

A comprehensive semen analysis following the World Health Organization guidelines (5) is fundamental at the diagnosing of reproductive potential and the selection of appropriate clinical management. Unfortunately, about 30% of infertile men are diagnosed by idiopathic oligoasthenoteratozoospermia (iOAT) after semen analysis (6). iOAT is a complex medical disorder, in which sperm count, motility and morphology are impaired. Until recently, the cause of iOAT is unknown and cannot be diagnosed using the currently available laboratory methods (7). Treatment of iOAT is a problematic, yet no supporting evidence is present for the variety of the available drugs and antioxidants (7). Although intracytoplasmic sperm injection (ICSI) has been proposed as a solution to overcome untreatable iOAT, impaired sperm quality negatively affects embryonic development and clinical outcomes (8, 9).

Eliminating or minimizing, even one adverse factor such as smoking, alcohol and stress, are thought to have a beneficial role on assisted reproductive technologies (ARTs) outcomes (10). To date, studies of the role of ejaculation frequency on ICSI outcomes among infertile men are lacking. In the current study, we prospectively evaluated the association between increasing the frequency of ejaculation and ICSI outcomes for iOAT male partners of couples undergoing ICSI.

Materials and methods

Overall study design

This is a prospective study held at a specialized fertility and gynecology center between November 2018 and September 2019. Approval by the institute's internal review board committee was obtained and all participants signed a written informed consent form before prior to the commencement of this study.

A detailed reproductive, medical and surgical history was taken from all male participants for evaluation, including developmental history, chronic medical illness, infections, surgical procedures, drugs and environmental exposures, lifestyle habitats, sexual history, and ARTs history. Two semen samples were analyzed before the beginning of treatment strategy to evaluate semen according to the World Health Organization (WHO) recommendation (5). Couples included in the present study met the following characteristics: 18-37 years female partners, normal uterus as observed by transvaginal ultrasound, male partners suffered from severe male factor cases; defined in our study as (count>5x106/ml, motility≥30%, progressive motility ≥5 %, abnormal forms ≥96), with ejaculation frequency <6 times per month. Patients with previously achieved pregnancy after ICSI, with frozen or non-ejaculated spermatozoa, and patients

enrolled in pre-genetic diagnosis program were excluded from the analysis. Women were also excluded if they had endometriosis or poor endometrium (<8 mm diameter) on the hCG trigger day. We excluded cases of OAT that have abnormal endocrine function (serum testosterone, inhibin, estradiol, LH and FSH levels), infection (White blood cells >1x106/ml), presence of discernable cause for their subfertile status.

With respect to the two inclusion cohorts, eighty-one patients turned out that patients were randomly distributed into two groups (Intervention group, n=44; Control group, n=37). Male partners in the intervention group were instructed to change the lifestyle by increasing the ejaculation frequency for one month before ICSI (three times per week) and prescribed antioxidant therapy (L-Carnitine (2g daily; Carnivita forte, EVA Pharma, Egypt), vitamin C (1g daily; vitacid C, Cid. Giza, Egypt), and vitamin E(400mg daily; Pharco, Egypt) for 3 months before ICSI. Male patients in the control group received a 3 months treatment of antioxidants only (L-Carnitine (2g daily), vitamin C (1g daily), and vitamin E (400mg daily).

ICSI treatment

All women underwent ovarian stimulation with agonist GnRH analogs according to our standard protocols (11). When two or more follicles were ≥ 18 mm, recombinant human chorionic gonadotropin (rhCG; Ovitrelle®, Serono, Geneva, Switzerland) was administered. Oocyte retrieval was performed 36 hours after the administration of rhCG with transvaginal ultrasound guidance. Two hours later, denudation was performed and only metaphase II stage oocytes (MII) were injected using fresh sperm ejaculates according to (12).

Fertilization check was carried out 16-18 hours after injection and oocytes with two pronuclei were considered as normally fertilized. The embryos were then cultured to the blastocyst stage. Forty-eight hours after ICSI embryos were scored for quality according to a system that takes into account the number of blastomeres, the degree of fragmentation, the symmetry of the blastomeres, the presence of multinucleation, and the compaction status according to the Istanbul consensus (13). Top-quality cleaved embryos were defined as 7–8 cells on day 3, with symmetric and uninucleated blastomeres and <10% fragmentation by volume. On day 5, blastocysts were graded using Gardner and Schoolcraft grading system (14). Top-quality blastocysts were identified as expanded day 5 blastocysts (>3), with rounded and dense inner cell mass and many twin trophectoderm cells creating a connected zone. The embryos were transferred into the uterus at the day 5 blastocyst stage using an embryo transfer catheter (Labotect, Göttingen, Germany) under ultrasound guidance.

Luteal support was initiated after retrieval with vaginal progesterone suppositories twice daily (Cyclogest 400 mg, Actavis, Barnstaple,UK, Ltd.) and continued until a negative pregnancy test or until 8 weeks' gestation. A serum β-hCG test was performed approximately 2 weeks after embryo transfer to confirm a pregnancy. A clinical pregnancy was defined as the presence of a fetal heartbeat on ultrasound scan 4 weeks or more after ET. A pregnancy scan was performed between >20 weeks' gestation to identify ongoing pregnancies.

Study end points

The primary outcome was the rate of top-quality blastocysts (≥3.1.1 formed blastocysts per fertilized oocyte). Secondary outcomes were fertilization rate (the number of normally fertilized oocytes at 16–18 h after ICSI/number of injected oocytes), embryo cleavage rate (the number of cleaved zygotes/number of fertilized oocytes) and blastocyst formation rate, defined as the number of cleaved zygotes per number of fertilized oocytes. Other outcome measures included the rates of biochemical pregnancy, clinical pregnancy, ongoing pregnancy, and implantation (the number of gestational sacs

observed divided by the number of embryos transferred).

Sample Size and statistical analysis

Sample-size calculation was based on the observed differences in top-quality blastocysts from existing data in the center in which the study was conducted, which was shown to be increasing the rate of top-quality blastocysts from 20% to 40. For this difference of 20%, with a power of 95% and an alpha of 5%, 400 oocytes needed to be recruited into each arm. Assuming and adjusting for a worst-case scenario of 10% drop out, 440 oocytes needed to be recruited into each arm; making 880 oocytes the overall required sample size for the study.

Data were entered into the Statistical Package for the Social Sciences program (SPSS), version 20, to be statistically analyzed. Continuous variables were summarized as means with SDs. Dichotomous data were reported as percentages. The odds ratio and 95% confidence interval were calculated. A P value of <.05 was considered statistically significant.

Results

The majority of patients were experiencing their first IVF cycle (61.7%), whereas 24.7% had previously undergone one failed, and 13.6% had undergone two failed ICSI cycles.

Demographics and cycle characteristics

No significant differences between both groups in terms of ages of the women, BMI, duration of time attempting to conceive, number of previous IVF/ICSI attempts, basal FSH (IU/L), antral follicular count, days of stimulation, total FSH/HMG, estradiol level, progesterone level, number of oocyte collected, maturity rate, or number of embryo transferred was detected (Table 1). Furthermore, there were no significant differences observed between the males' demographics and semen parameters between both groups, as detailed in Table 2.

Embryological outcomes and laboratory performance

Embryological outcomes of both groups are presented in Table 3. The rates of fertilization, cleavage and blastocyst formation were similar in the interventional and control groups. The quality of all top cleaved embryos on day 3 in the interventional group had significantly higher quality than those in the control group (74.4% vs. 59.4%; P=0.0001). Embryo compaction rate was also significantly increased in the interventional compared with the control group (36.8% vs. 18.1%; P<0.0001). Furthermore, In the interventional group there were 43% top-quality blastocyst per fertilized oocytes, whereas in the control group there was only 24.7% top-quality blastocyst per fertilized oocytes (P<0.0001; significant).

Clinical outcome measures

Clinical outcomes of both groups are presented in Table 3. The biochemical, clinical pregnancy and implantation rates were higher in the intervention group but were not statistically significant (biochemical pregnancy: intervention 59% vs. control 48.6% [RR 1.2; 95% CI 0.80- 1.83; P=0.35]; clinical pregnancy: intervention 52.3% vs. control 37.8% [RR 1.2; 95% CI 0.76- 1.92; P=0.42]; Implantation: intervention 37.8% vs. control 27.9% [RR 1.4; 95% CI 0.88-2.06; P=0.16]). Ongoing pregnancy rate was significantly higher in the intervention group (21/44), 47.7%) than in the control group (9/37, 24.3%; RR 1.96, 95% CI 1.03-3.75; P=0.04). The early pregnancy loss rate was 11.4% in the intervention group and 24.3% in control group, a difference that was not significant. Furthermore, the multiple pregnancy rate was (9/44, 20.5%) in the interventional group vs. (6/37, 16.2%) in the control group (22 of 180), resulting in no significant difference between both ET groups.

Discussion

In this prospective cohort study, we found that higher ejaculation frequency for one month before ICSI combined with antioxidant treatment for 3 months prior ICSI cycles were associated with statistically significantly higher top-quality cleaved embryos on day 3, compaction and top-quality blastocysts rates compared with antioxidant treatment only for 3 months in iOAT. We did observe differences in the rates of biochemical pregnancy (~10%), clinical pregnancy (~12%) as well as implantation (~10%) in favor to intervention group. However, these remarkable differences failed to detect statistical significance because of our limited small sample size. Moreover, our results showed a significant increase in ongoing pregnancy rate in intervention group.

iOAT has been attributed to increase of reactive oxygen species (ROS) in the tubules and seminal plasma with a reduce in total antioxidant capacity. This may cause apoptosis and consequently affecting semen parameters (15). Undeniably, excess ROS generation negatively affects the outcome of assisted reproduction, leading to lower fertilization, implantation as well as pregnancy rates (16). Methods to treat iOAT are scarce and controversial and mainly based on elevating excessive ROS. Pharmacotherapy of oral supplementation with antioxidants is promising in decreasing ROS, improving semen parameters and ART outcomes of subfertile men suffering from iOAT. Patients included in this study were supplemented with L-carnitine, Vitamin C and vitamin E as a combinational antioxidant for 3 months. A 3-month period of treatment was chosen in our study to allow for a full cycle of spermatogenesis

Multiple confounding factors such as frequency of ejaculation, abstinence time, excessive heat exposure and obesity act as potential sources of ROS and should be considered and modified as possible. Although there is a paucity of information about the effect of lifestyle modification on semen parameters and ART outcomes, it thought to be beneficial without risk in men with iOAT. Little attention has been paid to the effects of ejaculation frequency on fertility. A low frequency of ejaculation may be an important cause of impaired male reproductive function (17). Increased frequency of ejaculation was observed to be associated lower oxidative stress exposure on sperm and could overcome the adverse effect of other lifestyle (18) as well as iOAT (19) was observed to be related to daily ejaculation. On the basis of the results of our study, increasing ejaculation frequency was significantly associated with better embryo quality and ongoing pregnancies in ICSI cycles. This is in agreement with a previous preliminary report, which showed a significant increase in sperm vitality, embryonic development and the probability of subsequent pregnancy after ICSI among 3 infertile couples with high repeated ejaculation frequency in necrozoospermic males (20).

The apparent limitations of our study are mainly attributed to its nature, being prospective non-randomized study with small sample size to detect difference in clinical outcomes and the lack of blinding. Furthermore, the data of live-birth and perinatal outcomes were not available for the entire cohort. The study also included only iOAT patients, which limits the generalizability of the study findings. Therefore, large scale multicenter randomized controlled studies are required to confirm and validate our findings.

In conclusion, our results suggest that increasing the frequency of ejaculation may be an effective option when combined with antioxidant therapy for iOAT treatment. Although the conclusions reached in terms of top-quality cleaved, compaction, top-quality blastocysts and ongoing pregnancy are validated by an adequate statistical power; further additional studies with larger sample size are encouraged to validate our results.

Conflicts of interest

None declared.

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Table I: Baseline and clinical outcomes of both groups

Variable	Intervention group (n=44)	Control group (n=37)	95% CI
Mean female age (Years)	30.6±4.12	30.1±4.18	-0.5(-2.34-1.34)
Mean female BMI (Kg/m^2)	27.02±2.5	26.97±3.0	0.05(-1.26-1.16)
Mean duration of infertility (Years)	6.0±3.1	5.7±2.5	-0.3(-1.56-0.96)
Previous ICSI /IVF attempts	1.8±1.2	1.7±0.8	-0.1(-0.56-0.36)
Etiology Male Combined	 (28/44) 63.6% (16/44) 36.4%	 (23/37) 62.2% (14/37) 37.8%	1.4 %(-20.7-23.7)
Basal FSH (mIU/mL)	6.9±1.1	6.5±0.9	-0.4(-0.85-0.05)
AFC	15.8±5.7	14.4±5.8	-1.4(-3.95-1.15)
Days of stimulation	10.7±0.9	11.0±0.9	0.3(-0.1-0.7)
Total dose of gonadotropin (IU)	2629.4±666.7	2749.3±634.4	119.9(-169.66-409.46)
E2 trigger (pg/ml)	2667.7±799.4	2413.3±902.7	-254.4(-630.91-122.11)
P4 trigger (ng/mL)	1.0±0.4	1.1±0.3	0.1(-0.06-0.26)
COC retrieved	13.8±5.3	14.2±3.2	0.4(-1.58-2.38)
MII injected	12.1±4.8	11.9±3.3	-0.2(-2.06-1.66)
Mean No. of embryos transferred	2.2±0.5	2.3±0.5	0.1(-0.12-0.32)
Mean endometrial thickness (mm) on ET day	11.8±2.0	12.1±1.5	0.3(-0.49-1.09)

Note: Values are mean ± SD or percentages.
P < 0.05 was considered to be significant when compared with the antioxidant only control group.
ET= **embryo transfer**; BMI= **body mass index**; ICSI= **intracytoplasmic sperm injection**; E2= **estradiol**; COC= **cumulus corona cell oocyte complexes**; FSH= **follicle-stimulating hormone**; AFC =**Antral follicle count**; P4= **progesterone**.